

WE CLAIM:

1. A method for expanding TcR $\gamma\delta^+$ T cells in a starting sample comprising:

- (1) culturing cells in the starting sample in a first culture medium comprising (a) a T cell mitogen, (b) interleukin-2 and (c) interleukin-4; and
- (2) culturing the cells obtained in step (1) in a second culture medium comprising (i) interleukin-2 and (ii) interleukin-4 to expand TcR $\gamma\delta^+$ T cells.

2. A method for expanding TcR $\gamma\delta^+$ T cells in a starting sample comprising:

- (1) culturing cells in the starting sample in a first culture medium comprising XLCM; and
- (2) culturing the cells obtained in step (1) in a second culture medium comprising (i) interleukin-2 and (ii) interleukin-4 to expand TcR $\gamma\delta^+$ T cells.

3. A method according to claim 1 or 2 wherein the first and second culture media contain serum or plasma.

4. A method according to claim 1, 2 or 3 wherein prior to step (1) the cells in the starting sample are enriched for T cells.

5. A method according to any one of claims 1 to 4 wherein prior to step (1) the cells in the starting sample are enriched for CD4 $^+$ cells.

6. A method according to any one of claims 1 to 5 wherein prior to step (1) the cells in the starting sample are depleted of CD14 $^+$, CD16 $^+$, CD19 $^+$, CD56 $^+$ and glycophorin A $^+$ cells.

AMENDED SHEET

IPEA/EP

7. A method according to any one of claims 1 to 6 wherein prior to step (1) the cells in the starting sample are depleted of TcR $\alpha\beta$ ⁺ T cells.

8. A method according to any one of claims 1 to 7 wherein prior to step (1) the cells in the starting sample are depleted of non-TcR $\gamma\delta$ ⁺ T cells.

9. A method according to any one of claims 1 to 8 wherein the starting sample is selected from peripheral blood, bone marrow, lymphoid tissue, epithelia, thymus, liver, spleen, cancerous tissue, infected tissue, lymph node tissue or fractions thereof.

10. A method according to claim 9 wherein the starting sample is human peripheral blood or a fraction thereof.

11. A method according to any one of claims 1 to 10 wherein the starting sample is low density mononuclear cells.

12. A method according to any one of claims 1 or 3 to 10 wherein in the first culture medium the T cell mitogen is present in an amount from about 0.01 to about 100 $\mu\text{g/ml}$; the IL-2 is present in an amount from about 0.1 to about 1000 ng/ml and the IL-4 is present in an amount from about 0.1 to about 1000 ng/ml.

13. A method according to any one of claims 1 or 3 to 10 wherein in the first culture medium the T cell mitogen is present in an amount from about 0.1 to about 50 $\mu\text{g/ml}$; the IL-2 is present in an amount from about 1 to about 100 ng/ml and the IL-4 is present in an amount from about 1 to about 100 ng/ml.

AMENDED SHEET
IPEA/EP

14. A method according to any one of claims 1 or 3 to 10 wherein in the first culture medium the T cell mitogen is present in an amount from about 0.5 to about 10 µg/ml; the IL-2 is present in an amount from about 2 to about 50 ng/ml and the IL-4 is present in an amount from about 2 to about 50 ng/ml.

15. A method according to any one of claims 1 or 3 to 14 wherein the first culture medium comprises 1µg/mL of a T cell mitogen; 10 ng/mL IL-2 and 10 ng/mL IL-4.

16. A method according to any one of claims 1 or 3 to 14 wherein the T cell mitogen is concanavalin A.

17. A method according to claim 3 wherein the serum or plasma is present in an amount from about 1 to about 25% by volume.

18. A method according to claim 3 wherein the serum or plasma is present in an amount from about 2 to about 20% by volume.

19. A method according to claim 3 wherein the serum or plasma is present in an amount from about 2.5 to about 10% by volume.

20. A method according to claim 3 wherein the serum or plasma is present in an amount of about 5% by volume.

21. A method according to any one of claims 1 to 20 wherein in the second culture medium the IL-2 is present in an amount from about 0.1 to about 1000 ng/ml and the IL-4 is present in an amount from about 0.1 to about 1000 ng/ml.

- sub
B2
- (3) culturing the cells obtained in step (2) in a first culture medium comprising (a) a T cell mitogen, (b) interleukin-2 and (c) interleukin-4; and
- (4) culturing the cells obtained in step (3) in a second culture medium comprising (i) interleukin-2 and (ii) interleukin-4 to expand TcR $\gamma\delta^+$ T cells.

5

30. A method according to claim 29 wherein step (2) additionally comprises depleting the cells of CD14 $^+$, CD16 $^+$, CD19 $^+$, CD56 $^+$ and glycophorin A $^+$ cells.

10 31. A method according to claim 29 wherein step (2) additionally comprises depleting the cells of TcR $\alpha\beta^+$ T cells.

32. A cell preparation enriched in TcR $\gamma\delta^+$ T cells prepared according to the method of any one of claims 1 to 28.

33. A cell preparation enriched in TcR $\gamma\delta^+$ T cells according to claim

15 32 wherein greater than 70% of the total cells are TcR $\gamma\delta^+$ T cells.

34. A cell preparation according to claims 32 or 33 wherein greater than 80% of the total cells are TcR $\gamma\delta^+$ T cells.

35. A cell preparation according to claims 32, 33 or 34 wherein greater than 90% of the total cells are TcR $\gamma\delta^+$ T cells.

20 36. A cell preparation according to any one of claims 32 to 35 which comprises V δ 1 $^+$ and V δ 2 $^+$ TcR $\gamma\delta^+$ T cells.

AMENDED SHEET
IPEA/EP
37

22. A method according to any one of claims 1 to 20 wherein in the second culture medium the IL-2 is present in an amount from about 1 to about 100 ng/ml and the IL-4 is present in an amount from about 1 to about 100 ng/ml.

23. A method according to any one of claims 1 to 20 wherein in the second culture medium the IL-2 is present in an amount from about 2 to about 50 ng/ml and the IL-4 is present in an amount from about 2 to about 50 ng/ml.

24. A method according to any one of claims 1 to 23 wherein the second culture medium comprises 10 ng/mL IL-2 and 10 ng/mL IL-4.

25. A method according to claim 2 wherein the XLCM is present in an amount from about 1 to about 25%.

26. A method according to claim 2 wherein the XLCM is present in an amount from about 2 to about 20%.

27. A method according to claim 2 wherein the XLCM is present in an amount from about 2.5 to about 10%.

28. A method according to claim 2 wherein the XLCM is present in an amount from about 5%.

29. A method for obtaining TcR $\gamma\delta^+$ T cells from a sample from a patient with chronic myelogenous leukemia comprising:

- (1) obtaining low density mononuclear cells (LDMNC) from the sample;
- (2) depleting the cells obtained in step (1) of CD33 $^+$ cells;

AMENDED SHEET

37. A cell preparation according to claim 36 which comprises about 50-90% V δ 1⁺ and about 10-50% V δ 2⁺ TcR $\gamma\delta$ ⁺ T cells of the total TcR $\gamma\delta$ ⁺ T cells in the preparation.

38. A cell preparation according to claim 36 which comprises about
5 70% V δ 1⁺ and about 30% V δ 2⁺ TcR $\gamma\delta$ ⁺ T cells of the total TcR $\gamma\delta$ ⁺ T cells in the preparation.

39. A use of a cell preparation according to any one of claims 32 to 38 to prepare a medicament to modulate an immune response.

40. A use of a cell preparation according to any one of claims 32 to
10 38 to prepare a medicament to treat an infection.

41. A use of a cell preparation according to any one of claims 32 to 38 to prepare a medicament to treat cancer.

42. A use of a cell preparation according to any one of claims 32 to 38 to prepare a medicament to treat chronic myelogenous leukemia.

43. A use of a cell preparation according to any one of claims 32 to 38 to prepare a vaccine.

44. A method of modulating an immune response comprising administering an effective amount of TcR $\gamma\delta$ ⁺ T cells obtained according to the method of any one of claims 1 to 28 to an animal in need thereof.

45. A method for treating an infection comprising administering
20 an effective amount of TcR $\gamma\delta$ ⁺ T cells obtained according to the method of any one of claims 1 to 28 to an animal in need thereof.

AMENDED SHEET
IPEA/EP

47. A method for treating chronic myelogenous leukemia comprising administering an effective amount of TcR $\gamma\delta^+$ T cells obtained according to the method of any one of claims 1 to 28 to an animal in need thereof.

SECRET

AMENDED SHEET
IPEA/EP